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CONTENTS/SUMMARIES

Summary: We present a collection of 182 isogenic strains containing genetically linked antibiotic resistance elements located at approximately 1-min intervals around the Escherichia coli chromosome. At most positions both Tn10 (Tet^r) and TN10kan (Kan^r) elements are available, so that the collection contains a linked set of alternating antibiotic resistance markers. The map position of each insertion has been aligned to the E. coli genetic map as well as to the Kohara ordered clone bank. These strains are designed to be used in a rapid two-step mapping system in E. coli. In the first step, the mutation is localized to a 5- to 15-min region of the chromosome by Hfr mapping with a set of Hfr strains containing either Tn10 or Tn10kan elements located 20 min from their respective origins of transfer. In the second step, the mutation is localized to a 1-min region by P1 transduction, with a collection of isogenic insertion strains as donors. We discuss the uses of this collection of strains to map and eventually to clone a variety of mutations in E. coli.

Summary: Until 10 years ago, R bodies were known only as diagnostic features by which endosymbionts of paramecia were identified as kappa particles. They were thought to be limited to the cytoplasm of two species in the Paramecium aurelia species complex. Now, R bodies have been found in free-living bacteria and other Paramecium species. The organisms now known to form R bodies include the cytoplasmic kappa endosymbionts of P. biaurelia and P. tetraurelia, the macronuclear kappa endosymbionts of P. caudatum, Pseudomonas avenae (a free-living plant pathogen), Pseudomonas taeniospiralis (a hydrogen-oxidizing soil microorganism), Rhodospirillum centenum (a photosynthetic bacterium), and a soil bacterium, EPS-5028, which is probably a pseudomonad. R bodies themselves fall into five distinct groups, distinguished by size, the morphology of the R-body ribbons, and the unrolling behavior of wound R bodies. In recent years, the inherent difficulties in studying the organization and assembly of R bodies by the obligate endosymbiont kappa, have been alleviated by cloning and expressing genetic determinants for these R bodies (type 51) in Escherichia coli. Type

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51 R-body synthesis requires three low-molecular-mass polypeptides. One of these is modified posttranslationally, giving rise to 12 polypeptide species, which are the major structural subunits of the R body. R bodies are encoded in kappa species by extrachromosomal elements. Type 51 R bodies, produced in Caedibacter taeniospiralis, are encoded by a plasmid, whereas bacteriophage genomes probably control R-body synthesis in other kappa species. However, there is no evidence that either bacteriophages or plasmids are present in P. avenae or P. taeniospiralis. No sequence homology was detected between type 51 R-body-encoding DNA and DNA from any R-body-producing species, except C. varicaedens 1038. The evolutionary relatedness of different types of R bodies remains unknown.

Summary: Ammonia oxidizers (family Nitrobacteraceae) and methanotrophs (family Methylococcaceae) oxidize CO and CH_4 to CO_2 and NH_4^+ to NO_2^-. However, the relative contributions of the two groups of organisms to the metabolism of CO, CH_4, and NH_4^+ in various environments are not known. In the ammonia oxidizers, ammonia monooxygenase, the enzyme responsible for the conversion of NH_4^+ to NH_2OH, also catalyzes the oxidation of CH_4 to CH_3OH. Ammonia monooxygenase also mediates the transformation of CH_3OH to CO_2 and cell carbon, but the pathway by which this is done is not known. At least one species of ammonia oxidizer, Nitrosococcus oceanus, exhibits a K_m for CH_4 oxidation similar to that of methanotrophs. However, the highest rate of CH_4 oxidation recorded in an ammonia oxidizer is still five times lower than rates in methanotrophs, and ammonia oxidizers are aparently unable to grow on CH_4. Methanotrophs oxidize NH_4^+ to NH_2OH via methane monooxygenase and NH_4^+ to NH_2OH via methane monooxygenase and NH_2OH to NO_2^- via an NH_2OH oxidase which may resemble the enzyme found in ammonia oxidizers. Maximum rates of NH_4^+ oxidation are considerably lower than in ammonia oxidizers, and the affinity for NH_4^+ is generally lower than in ammonia oxidizers. NH_4^+ does not apparently support growth in methanotrophs. Both ammonia monooxygenase and methane monooxygenase oxidize CO to CO_2, but CO cannot support growth in either ammonia oxidizers or methanotrophs. These organisms have affinities for CO which are comparable to those for their growth substrates and often higher than those in carboxydobacteria. The methane monooxygenases of methanotrophs exist in two forms: a soluble form and a particulate form. The soluble form is well characterized and appears unrelated to the particulate. Ammonia monooxygenase and the particulate methane monooxygenase share a number of similarities. Both enzymes contain copper and are membrane bound. They oxidize a variety of inorganic and organic compounds, and their inhibitor profiles are similar. Inhibitors thought to be specific to ammonia oxidizers have been used in environmental studies of nitrification. However, almost all of the numerous compounds found to inhibit ammonia oxidizers also inhibit methanotrophs, and most of the inhibitors act upon the monooxygenases. Many probably exert their effect by chelating copper, which is essential to the proper functioning of some monooxygenases. The lack of inhibitors specific for one or the other of the two groups of bacteria hampers the determination of their relative roles in nature.

Summary: Microbial ureases hydrolyze urea to ammonia and carbon dioxide. Urease activity of an infectious microorganism can contribute to the development of urinary stones, pyelonephritis, gastric ulceration, and other diseases. In contrast to these harmful effects, urease activity of ruminal and gastrointestinal microorganisms can benefit both the microbe and host by recycling (thereby conserving) urea nitrogen. Microbial ureases also play an important role in utilization of environmental nitrogenous compounds and urea-based fertilizers. Urease is a high-molecular-weight, multimeric, nickel-containing enzyme. Its cytoplasmic location requires that urea enter the cell for utilization, and in some species energy-dependent urea uptake systems have

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been detected. Eucaryotic microorganisms possess a homopolymeric urease, analogous to the well-studied plant enzyme composed of six identical subunits. Gram-positive bacteria may also possess homopolymeric ureases, but the evidence for this is not conclusive. In contrast, ureases from gram-negative bacteria studied thus far clearly possess three distinct subunits with M_rs of 65,000 to 73,000 (α), 10,000 to 12,000 (β), and 8,000 to 10,000 (γ). Tightly bound nickel is present in all ureases and appears to participate in catalysis. Urease genes have been cloned from several species, and nickel-containing recombinant ureases have been characterized. Three structural genes are transcribed on a single messenger ribonucleic acid and translated in the order γ, β, and then α. In addition to these genes, several other peptides are encoded in the urease operon of some species. The roles for these other genes are not firmly established, but may involve regulation, urea transport, nickel transport, or nickel processing.

Summary: The bacterial phosphotransferase system (PTS) functions in a variety of regulatory capacities. One of the best characterized of these is the process by which the PTS regulates inducer uptake and catabolite repression. Early genetic and physiological evidence supported a mechanism whereby the phosphorylation state of an enzyme of the PTS, the enzyme III specific for glucose (III^{Glc}), allosterically inhibits the activities of a number of permeases and catabolic enzymes, the lactose, galactose, melibiose, and maltose permeases, as well as glycerol kinase. Extensive biochemical evidence now supports this model. Evidence is also available showing that substrate binding to those target proteins enhances their affinities for III^{Glc}. In the case of the lactose permease, this positively cooperative interaction represents a well documented example of transmembrane signaling, demonstrated both in vivo and in vitro. Although the PTS-mediated regulation of cyclic AMP synthesis (catabolite repression) is not as well defined from a mechanistic standpoint, a model involving allosteric activation of adenylate cyclase by phospho-III^{Glc}, together with the evidence supporting it, is presented. These regulatory mechanisms may prove to be operative in gram-positive as well as gram-negative bacteria, but the former organisms may have introduced variations on the theme by covalently attaching III^{Glc}-like moieties to some of the target permeases and catabolic enzymes. It appears likely that the general process of PTS-catalyzed protein phosphorylation-dephosphorylation will prove to be important to the regulation of numerous bacterial physiological processes, including chemotaxis, intermediary metabolism, gene transcription, and virulence.

Summary: The capacity of organisms to respond to fluctuations in their osmotic environments is an important physiological process that determines their abilities to thrive in a variety of habitats. The primary response of bacteria to exposure to a high osmotic environment is the accumulation of certain solutes, K^+, glutamate, trehalose, proline, and glycinebetaine, at concentrations that are proportional to the osmolarity of the medium. The supposed function of these solutes is to maintain the osmolarity of the cytoplasm at a value greater than the osmolarity of the medium and thus provide turgor pressure within the cells. Accumulation of these metabolites is accomplished by de novo synthesis or by uptake from the medium. Production of proteins that mediate accumulation or uptake of these metabolites is under osmotic control. This review is an account of the processes that mediate adaptation of bacteria to changes in their osmotic environment.

Summary: Transformation with exogenous deoxyribonucleic acid (DNA) now appears to be possible with all fungal species, or at least all that can be grown in culture. This

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field of research is at present dominated by Saccharomyces cerevisiae and two filamentous members of the class Ascomycetes, Aspergillus nidulans and Neurospora crassa, with substantial contributions also from fission yeast (Schizosaccharomyces pombe) and another filamentous member of the class Ascomycetes, Podospora anserina. However, transformation has been demonstrated, and will no doubt be extensively used, in representatives of most of the main fungal classes, including Phycomycetes, Basidiomycetes (the order Agaricales and Ustilago species), and a number of the Fungi Imperfecti. The list includes a number of plant pathogens, and transformation is likely to become important in the analysis of the molecular basis of pathogenicity. Transformation may be maintained either by using an autonomously replicating plasmid as a vehicle for the transforming DNA or through integration of the DNA into the chromosomes. In S. cerevisiae and other yeasts, a variety of autonomously replicating plasmids have been used successfully, some of them designed for use as shuttle vectors for Escherichia coli as well as for yeast transformation. Suitable plasmids are not yet available for use in filamentous fungi, in which stable transformation is dependent on chromosomal integration. In Saccharomyces cerevisiae, integration of transforming DNA is virtually always by homology; in filamentous fungi, in contrast, it occurs just as frequently at nonhomologous (ectopic) chromosomal sites. The main importance of transformation in fungi at present is in connection with gene cloning and the analysis of gene function. The most advanced work is being done with S. cerevisiae, in which the virtual restriction of stable DNA integration to homologous chromosome loci enables gene disruption and gene replacement to be carried out with greater precision and efficiency than is possible in other species that show a high proportion of DNA integration events at nonhomologous (ectopic) sites. With a little more trouble, however, the methodology pioneered for S. cerevisiae can be applied to other fungi too. Transformation of fungi with DNA constructs designed for high gene expression and efficient secretion of gene products appears to have great commercial potential.

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